UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA LISBOA

TRABALHO FINAL
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Clínica Universitária de Neurologia

Prognostic value of phrenic nerve conduction study in amyotrophic lateral sclerosis – a systematic review and meta-analysis

Cláudia Alexandra Santos da Silva

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Orientado por:

Mamede de Carvalho

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Resumo

A maioria das mortes causadas pela esclerose lateral amiotrófica (ELA) ocorrem como resultado de complicações respiratórias, o que significa que as provas de função respiratória servem como biomarcadores prognósticos nesta condição. O estudo da condução do nervo frénico (PNCS) é um teste rápido, não-volitivo e acessível que pode ser utilizado para a avaliação da função respiratória nos doentes com ELA, através da medição da componente motora do potencial de ação do nervo (CMAP).

O nosso objetivo foi aferir o valor prognóstico do estudo da condução do nervo frénico na sua capacidade de predizer o risco de mortalidade, assim como a sua correlação com a capacidade vital forçada (CVF), nos doentes com ELA.

Foi conduzida uma revisão sistemática e meta-análise que analisou oito estudos observacionais. Como indicador primário considerámos os *hazard ratios* para a mortalidade a diferentes limiares de amplitude de CMAP. Como indicador secundário considerámos a correlação entre a amplitude de CMAP e a CVF.

Na meta-análise, verificou-se que os doentes com amplitude de CMAP igual ou inferior a 0.4mV tinham uma probabilidade de morrer 2.021 vezes superior, durante o período estudado (IC 95%= 1.161 a 3.522; I^2 = 69.77%; 381 participantes). A amplitude de CMAP mostrou-se correlacionar positivamente com a CVF (coeficiente de correlação de 0.400; IC 95%= 0.226 a 0.550; I2= 69.77%; 381 participantes). Por outro lado, verificou-se uma correlação fraca negativa entre a latência de CMAP e a CVF (coeficiente de correlação de -0.235; IC 95% = 0.447 a -0.024; I^2=15.92%; 112 participantes).

Com grau moderado de evidência o nosso estudo indica que o estudo da condução do frénico pode ser considerado um marcador adicional da função respiratória, nos doentes com ELA, mas que mais investigação é necessária.

Palavras-chave: esclerose lateral amiotrófica; nervo frénico; revisão sistemática; meta-análise; prognóstico.

O Trabalho Final exprime a opinião do autor e não da FML.

Abstract

The main cause of death in amyotrophic lateral sclerosis (ALS) is related to respiratory complications, meaning respiratory function as evaluated by dedicated tests is a relevant prognostic biomarkers in ALS. Phrenic nerve conduction study (PNCS) is a quick, non-volitional, and inexpensive test that can be used to assess respiratory function in ALS patients by the measurement of phrenic compound muscle action potential (CMAP).

We aimed to ascertain the prognostic value of PNCS in predicting mortality risk in ALS, and to test its correlation with forced vital capacity (FVC).

A systematic review and meta-analysis examined eight observational studies. As primary outcome we considered the hazard ratios for mortality at different cut-off thresholds of the CMAP amplitude. As secondary outcomes we considered the correlation between the CMAP amplitude and latency with forced vital capacity (FVC).

In the pooled analysis, patients with CMAP amplitude equal or below 0.4mV are 2.021 more likely to die over the studied period (95%CI 1.161 to 3.522; I2=55.9%; 2 studies; 338 participants). Amplitude of CMAP showed a moderate positive correlation with FVC (correlation coefficient of 0.400, 95%CI= 0.226 to 0.550; I2=69.77%; 381 participants). On the other hand, there was a weak negative correlation between CMAP latency and FVC (correlation coefficient of -0.235; 95% confidence interval= -0.447 to -0.024; I2=15.92%; 112 participants).

This study gives moderate evidence in favour of PNCS as an additional marker of pulmonary function in ALS patients, but further research is necessary.

Key-words: amyotrophic lateral sclerosis; phrenic nerve; systematic review; meta-analysis; prognosis.

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Introduction

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disease that involves the upper and lower motor neurons, typically affecting the limb, bulbar, and respiratory muscles.[10]

Respiratory failure (RF) frequently arises in a late stage of the disease, although it can be the presenting feature in about 3% of the patients.[7] Patients characteristically die from hypoventilation[9] with hypoxaemia and hypercapnia, often precipitated by respiratory infection, aspiration pneumonia or bronchial impaction.[4] The median overall survival after symptom onset is 3 years.[25]

Accurate assessment of pulmonary function is critical to detect early abnormalities, in order to estimate prognosis, provide patient counselling, and make treatment decisions.

Although several tests that have been studied, there is no consensus on the best measure of pulmonary function in ALS.[21] Pulmonary function tests (PFT) are the standard technique used across most diseases, being non-invasive and widely available. Among these, forced vital capacity (FVC) seems to be the most useful measure in ALS management and research, as it can predict hypercapnia[35] and prognosis,[6; 9] being considered the 'gold-standard' in this context. However, PFT have notable limitations, namely that the technique depends on patient cooperation, which is disturbed in patients with less motivation, depression, or other behavioural changes, all of which are common in ALS.[26] Additionally, people with ALS with bulbar involvement may have facial weakness, which impairs an accurate assessment by PFT. Altogether this means that for this population the predictive ability of PFT are meaningfully compromised.[9; 16]

Phrenic nerve conduction study (PNCS) is an alternative technique that objectively measures diaphragm innervation. Crucially, PNCS does not depend on patient collaboration and can be used irrespective of whether patients have spinal or bulbar involvement. In PNCS the phrenic compound muscle action potential (CMAP) is recorded applying a percutaneous technique. The CMAP amplitude has been shown to be predictive of hypoventilation, as defined by a $PaCO_2 > 45$ mmHg,[19] and of survival in ALS patients.[30; 33] Nevertheless, like other electrophysiological techniques, PNCS is not

as widely available as PFT, is subject to technical pitfalls, and some patients with severe orthopnoea are unable to tolerate the technique.

The primary objective of this systematic review and meta-analysis was to ascertain the prognostic value of PNCS in ALS patients.

Methods

The protocol followed the PRISMA-P guidelines[23] and was registered at Prospero (CRD42017079438). Reporting followed the MOOSE[40] and PRISMA guidelines[18]. Statistical data reporting followed the SAMPL guidelines.[17]

Eligibility criteria

Types of studies

We considered observational studies that compared the results of the index test, PNCS, with the reference standard, PFT. We included studies in which data have been collected either prospectively or retrospectively from consecutive series of patients with ALS followed in any setting.

No restrictions were made based on a minimal quality standard, minimal sample sizes, number of diseased cases, language, publication status or data of publication.

Participants

We included adults with a diagnosis of definite or probable ALS, as defined by the modified *El Escorial* criteria[4] of all ages and in any setting. According to these criteria, definite ALS is settled on clinical evidence of upper motor unit (UMU) (increased or tonic tendon reflexes, spasticity, pseudo-bulbar features, Hoffmann reflex and extensor plantar response) as well as lower motor neuron (LMN) (weakness, wasting, fasciculations) signs in the bulbar region and at least two of the other spinal regions, or the presence of UMN and LMN signs in three spinal regions. Probable ALS is defined based on clinical evidence of UMN and LMN signs at least 2 regions, with some UMN signs necessarily rostral to the LMN signs. However, patients with LMN and UMN signs in one region and widespread signs of loss of motor units on electromyography in two or more regions were considered equivalent to probable category (probable-lab supported) as per revised El Escorial criteria. [17]

Information sources and search strategy

Electronic identification of reports was conducted in MEDLINE (via Ovid), EMBASE (via Ovid), and Web of Science, using the strategies outlined in Appendix 1 and Appendix 2, from inception until October 2017. Text words and database subject headings (for example MeSH and EMTREE) were used in our search strategy.

In order to identify additional published studies we checked the reference lists of studies included for full text revision and contact experts in the field. We excluded unpublished results, with the exception of conference proceedings providing that these provide sufficient data.

Study selection

Two independent review authors (CSS, FBR) performed the first selection based on title and abstract. Each author indentified the studies requiring full text review. All studies identified as potential eligible studies were subject to full text review (CSS, FBR). Disagreements were solved by discussion or by a third author (GD). Both procedures were performed with Covidence®.

Data extraction and management

Two independent reviewers (CSS, FBR) extracted data from the studies included in this review using a pre-piloted standardised electronic form. Disagreements were resolved by consensus or with the help of a third reviewer (GD). Another reviewer (GD) double-checked the extracted data for prognosis.

Outcomes and prioritisation

The primary outcome was the assessment of the prognostic value of PNCS in overall survival. According to the type of data available, this was assessed using one or more of:

- Pooled hazard ratios for mortality using different cut-off thresholds of the CMAP amplitude obtained by PNCS.

- Pooled mortality rates using different cut-off thresholds of the CMAP amplitude obtained by PNCS. Unfortunately no such data were available.

For the primary outcome, a subgroup analysis were to be performed according to the main ALS phenotypical expressions (providing that sufficient data is available), as well as a sensitivity analysis by excluding studies deemed to be at high risk of bias.

The secondary outcomes were the disease severity, based on the correlation between FVC, our index test, and the CMAP amplitude, as well as the correlation between FVC and CMAP latency.

Assessment of risk of bias

The risk of bias of included studies was evaluated independently by two review authors (CSS, FBR) using the QUIPS (Quality in Prognosis Studies) tool.[11; 12]

Six domains are critical for assessing biases sufficiently large to distort the findings of prognosis research: (1) study participation; (2) study attrition; (3) prognostic factor measurement; (4) outcome measurement; (5) study confounding; and (6) statistical analysis and reporting. For each domain, three to seven "prompting items" are used to rate the adequacy of reporting by a study as "yes", "partial", "no", or "unsure"; an overall rating for each domain is assigned as "high", "moderate", or "low" risk of bias. Disagreements were solved by discussion or with consultation of a third review author (GD) in case of persisting disagreement.

Statistical analysis and data synthesis

To pool hazard ratios, we used a random effects generic inverse-variance method.

To pool the effect sizes of correlations coefficients of two continuous variables, we first transformed the coefficient into Fisher's *z* according to formula 1. For the two studies [1; 15] that presented a simple (i.e. univariable) linear regression, the correlation coefficient, *r,* was calculated as the square root of the coefficient of determination R^2. The variance

of *z* was computed according with formula 2, and the standard error (*SE*) according to formula 3. The meta-analyses was conducted using random effect inverse-variance approach. The final results were back-transformed according to formula 4. [20]

1. $z = 0.5 \ln(\frac{1+r}{1-r})$

2. $v_z = \frac{1}{n-3}$

3. $SE_z = \sqrt{V_z}$

4. $r = \frac{e^{2z}-1}{e^{2z}+1}$

Figure 1. Formulas for pooled analysis of correlations of continuous variables. *z* = Fisher's score; *r* = correlation coefficient; v_z = variance of z; *n* = sample size; *SE* = standard error.

We assessed statistical heterogeneity with the I^2.[13] A p-value <0.05 was considered statistically significant. All statistical analyses were done using Stata® (College Station, TX) 15.0 software.

Confidence in cumulative evidence

We evaluated the quality of the evidence using the grading of recommendations assessment, development and evaluation (GRADE) working group methods extended to prognosis factor research.[14]

Our evaluation was based on five domains that may decrease quality: (1) study limitations; (2) inconsistency; (3) indirectness; (4) imprecision; and (5) publication bias; and two factors that may increase quality: (1) moderate or large effect size; and (2) exposure response gradient[14]:

(1) Study limitations - according with the QUIPS tool for the risk of bias, outcomes are rated as: (1) no serious limitations for studies with low risk of bias for most of the bias domains; (2) serious limitations for studies at moderate or unclear risk of bias for most of the bias domains; (3) very serious limitations for studies at high risk of bias with respect to almost all of the domains.

(2) Inconsistency - the quality of evidence can be downgraded if (1) the points of effect of the studies cross the line of no effect and their confidence intervals show minimal or no overlap; or (2) the I^2 is substantial (≥50%).

(3) Indirectness - the quality of evidence may be downgraded when: (1) the participant population; (2) the prognosis factor(s); and/or (3) the outcomes considered in the primary studies do not fully represent the review question defined in the systematic review.

(4) Imprecision - the quality of evidence may be downgraded if: (1) the sample size included in the meta-analysis is insufficient; and/or (2) there is no precise estimate of the effect size in the meta-analysis, due to an excessively wide confidence interval that overlaps the value of no effect and contain values implying that the prognostic factor is associated with protection or increased risk.

(5) Publication bias - it should be considered for downgrading, unless the prognostic factor has been investigated in a large number of cohort studies.

And two factors that may increase quality:

(1) Moderate or large effect size – if there is a moderate or large pooled effect of the meta-analysis.
(2) Exposure response gradient – exists when elevated levels of prognostic factor (amount, longevity, intensity, etc) lead to a larger effect size over lower levels of the factor.

The overall quality or research evidence was defined as ‘high’, ‘moderate’, ‘low’ or ‘very low’

Results

Study selection

A total of 345 references (MEDLINE 120, EMBASE 170, and Web of Science 55) were retrieved through the electronic search (Figure 2). After automatic and manual deduplication, 233 references had their title and abstract screened. Of these, we excluded 204 non-illegible references. The full- texts of 29 references were examined in detail. Of these, 21 studies were excluded, 12 due to wrong study design, 4 due to duplication, 3 due to wrong outcomes, 1 due to insufficient information after contacting the authors. Eight studies met the inclusion criteria and were included (Figure 2). No additional records were obtained.

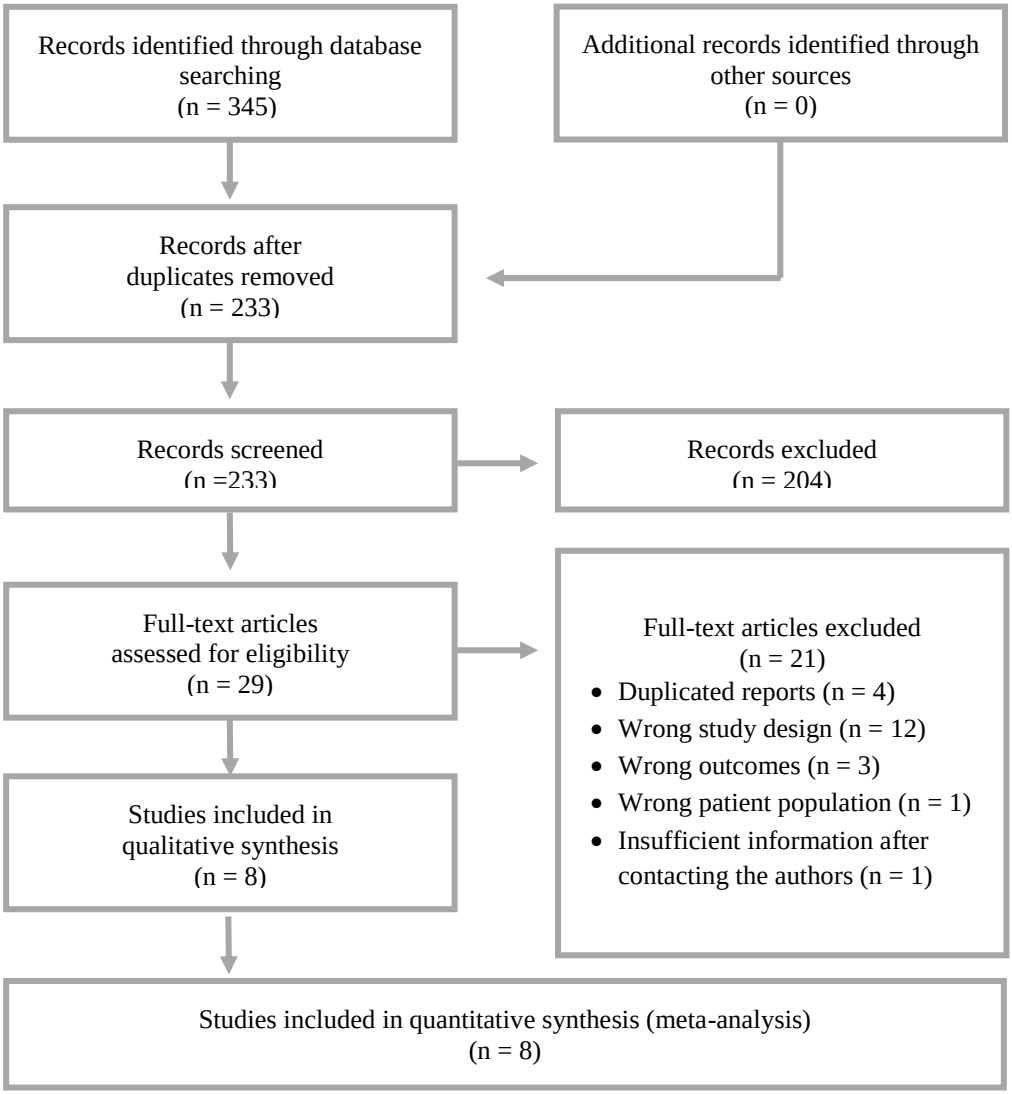

Figure 2. Study flow diagram of included studies.

Study characteristics

Methods

All included studies were observational: one was cross-sectional,[28] four were prospective cohorts,[15; 29; 39; 41] one was retrospective cohort,[33] and one was a case-control.[38] For Bokuda *et al*[1] only the abstract was available and it was not possible to assess if the cohort was either retrospective or prospective. All were published in English, single-centre, and set at tertiary hospitals. Three of these studies took place in Portugal, two in India, two in Japan, and one in the USA.

Participants

The included studies involved a total of 604 participants. The main inclusion criteria entailed adults over 18 years old, able to give informed consent, with a diagnosis of definite or probable ALS, as defined by the modified *El Escorial* criteria. Exclusion criteria included patients with implantable pacemakers, cardiac insufficiency, lung disease, polyneuropathy, diabetes mellitus, dementia, or malignancy. The participants' mean age ranged between 58.2 and 61.5 years, with a mean disease-duration between 16.0 and 27.6 months. Bulbar involvement ranged between 22,5% and 51,16% of the participants. In two studies[15; 41] participants initiated non-invasive ventilation during the study.

Index and reference tests

All patients underwent FPT. From these, forced vital capacity (FVC) was assessed by spirometry in sitting position, and expressed in percentage of the predicted lung capacity adjusted for gender, weight, height and race.

Phrenic nerve conduction studies techniques were comparable across studies. They were performed with percutaneous bipolar electrical stimulation of the phrenic nerve at the neck level (posterior to the lateral border of the sternocleidomastoid muscle, with the exception of one study,[41] in which stimulation was at applied at the supraclavicular fossa) and recorded through surface electrodes on xiphoid process (active) and costal margin of the mid clavicle (inactive). The ground electrode was placed over the sternum or ipsilateral arm. The latency of the diaphragmatic compound muscle action potentials (CMAP) was measured from the stimulus to the onset of potentials and expressed as

milliseconds (ms). The peak-to-peak amplitude of muscle action potentials were determined, and expressed as micro Volt (μV).

Clinical evaluation was performed through the ALS-Functional Rating Scale-Revised (ALS-FRS-R), including the respiratory subscore (0-12, which includes dyspnea, orthpopnea, need of ventilarory assistance).

Study, year of publication	Country	Study design	N	Age (years) Mean ± SD (range)	Gender Male N (%)	Disease duration (months) Mean ± SD (range)	ALSFSR-R Mean ±SD (range)	Bulbar onset N (%)	NIV
Bokuda 2014	Japan	Longitudinal	84	N/S	N/S	N/S	N/S	N/S	N/S
Jenkins 2016	USA	Longitudinal prospective	74	60.8 (34-84)	32 (43)	23.9 (2–102)	32.5 (8–45)	31 (31.0)	Yes
Pinto 2016	Portugal	Cross-sectional	42	58.4± 11.1 (34-77)*	20 (47.6)	17.8 ± 13.6 (5-58)	34.98 ± 3.1 (27–39)	11 (26.2)	No
Pinto 2017	Portugal	Longitudinal prospective	40	58.2 ± 10 (37- 77)*	20 (50.0)	16.0 ± 11.85	35.03 ± 3.40	9 (22.5)	No
Pinto 2012	Portugal	Longitudinal retrospective	254	60.9 ± 11.2 (28-80)	132 (52.0)	N/S	31.3 ± 5.6 (13–40)	79 (31.1)	No
Sathyaprabha 2010	India	Case-control	29	51.4 ±10.7 (30–68)	20 (69.0)	27.6 ± 34.3	N/S	8 (27.6)	No
Singh 2011	India	Longitudinal prospective	43	49.7±14.9	32 (74.4)	16.3 ± 15.7	44	18 (41.9)	No
Yamauchi 2014	Japan	Longitudinal prospective	43	61.5± 12.83	21 (48.8)	16.4 ± 9.8	31.77± 7.22	51.16	Yes

Table 1. Studies characteristics. N= number of participants; N/S= not specified; *Age at onset; ALSFRSR= amyotrophic lateral sclerosis functional rating scale-revised; NIV=non-invasive ventilation.

Outcomes

Three studies reported the hazard ratios for mortality.[1; 15; 34] However, one of these did not contained the enough information to be included in the analysis.[15]

Seven studies correlated the amplitude of CMAP with FVC.[1; 15; 28; 29; 38; 39; 41] Three studies correlated the latency of CMAP and FVC.[29; 38; 39] For the Pinto *et al* 2017[29] study, FVC and CMAP amplitude, and FVC and CMAP latency correlation coefficients were obtained through contact with the authors.

Synthesis of results

In the pooled analysis, participants with CMAP amplitude equal or below 0.4mV are 2.021 more likely to die over the studied period (95%CI 1.161 to 3.522; I^2=55.9%; 2 studies; 338 participants).

In the pooled analysis, amplitude of CMAP showed a moderate[5] positive correlation with FVC (r=0.400, 95%CI= 0.226 to 0.550; I^2=69.77%, 7 studies, 381 participants). On the other hand, there was a weak[5] negative correlation between latency of CMAP and FVC (r=- 0.235; 95%CI= -0.447 to -0.024; I^2=15.92%; 3 studies; 112 participants)

Results of individuals studies were incorporated in forest plots (see Figures 3, 4 and 5).

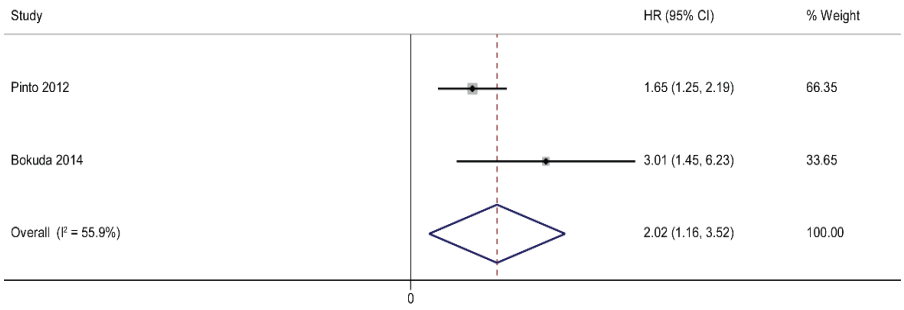

Figure 3. Pooled hazard ratios of mortality according with amplitude of CMAP of 0.4mV.

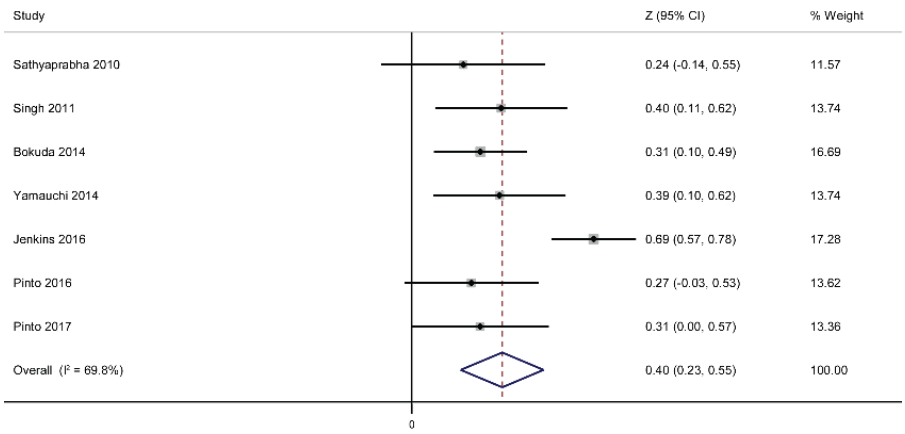

Figure 4. Pooled correlation coefficients between CMAP amplitude and FVC.

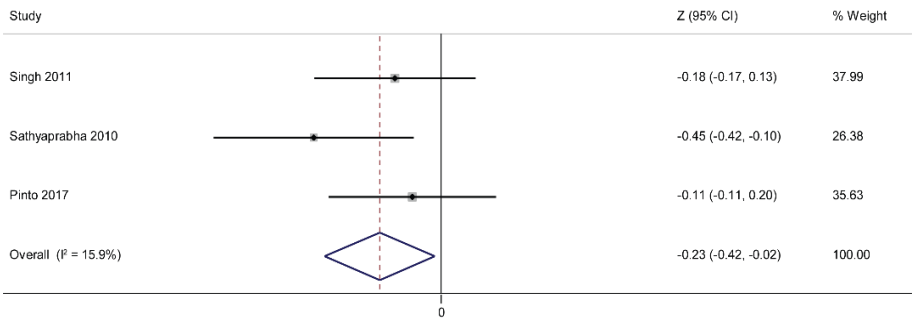

Figure 5. Pooled correlation coefficients between CMAP latency and FVC.

Risk of bias across studies

The overall risk of bias across studies was moderate to high (Figure 6).

For study participation the risk of bias was moderate, with high risk in one study (Bokuda et al 2014[1]) due the lack of information, and five studies with a moderate risk.[28; 29; 38; 39; 41] The information about the adequacy of study participation by eligible individuals was not available in none of the eight included studies. The period [1; 28; 29] and the place of recruitment [1; 29; 41] were not described accordingly in three studies.

The risk of study attrition bias was high, mainly because none of the included studies reported information about the participants who drop-out/were lost to follow-up.

The prognostic factor measurement item presented a moderate risk of bias. None of the longitudinal studies included reported a method to incorporate missing data, and the proportion of the data on prognostic factor available for analysis was unsure in two studies.[1; 29]

The risk of bias concerning outcome measurement was low. Nevertheless, method and setting of outcome measurement was not the same in all participants in Jenkins *et al* 2016,[15] as the stimulation intensity of the CMAP record was different in some of the included patients in the study. It was not clear if the method and setting of outcome measurement was the same in all participants in Singh *et al* 2011,[39] as the poor-quality of the spirograms in bulbar patients lead to a week of training by this subgroup of patients.

The study confounding risk of bias was high, as none of the eight included studies incorporated all possible confounders (i.e. respiratory symptoms, type of onset, ALSFRS-R, use of NIV) in the study design or data analysis.

Finally, the risk of bias concerning the study analysis and reporting was high, as it was not clear if the conceptual model framework or the statistical analysis of the eight studies were appropriate for the design of our study. Only two studies (Bokuda *et al* 2014,[1] Pinto *et al* 2012[33]) reported a Kaplan-Meier analysis for evaluation of the mortality related with PNCS.

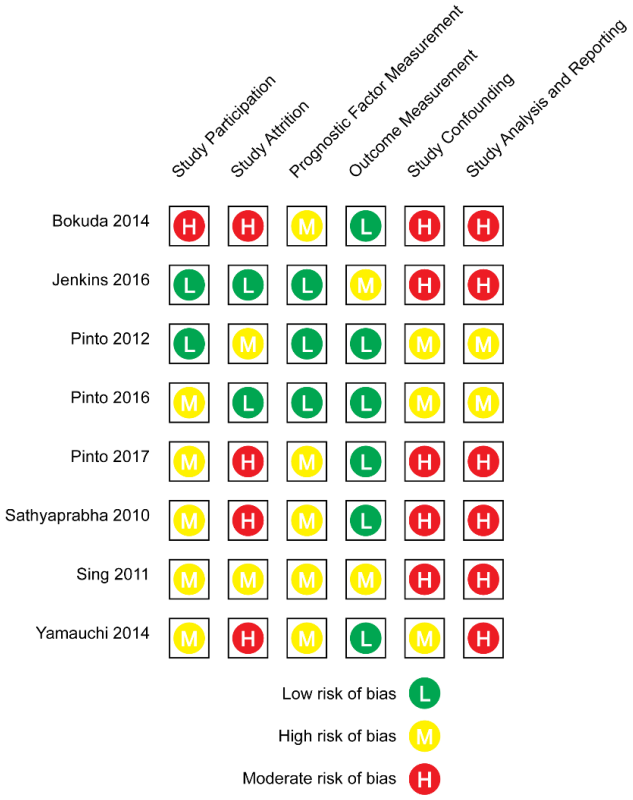

Figure 6. Risk of bias summary.

GRADE Quality of evidence

The overall quality of evidence was low to moderate. Table 2 details the GRADE approach to the quality of the available evidence.

PNCS probably predicts mortality. We found a moderate confidence that PNCS is associated with 102% relative increase in predicting mortality.

For all three outcomes, the quality of evidence was downgraded due to the fact that the evidence comes from studies with moderate to high risk of bias in the majority of the

domains. For mortality and correlation between FVC and CMAP amplitude, the quality of evidence was downgraded due to a significant heterogeneity across the studies, ascertained in the pooled analysis (I^2 = 55.9% and I^2= 69.77%, respectively). Additionally, for the correlation between FVC and CMAP latency, the quality of evidence was downgraded due to insufficient sample size included in the meta-analysis (Nº of participants = 112).The quality of evidence was upgraded for mortality due to high effect size in the meta-analysis (HR 2.02).

Outcome **№ of participants (studies)**	**Relative effect (95% CI)**	**Certainty**	**What happens**
Mortality № of participants: 388 (2 studies)	HR 2.02 (1.16 to 3.52)	⊕⊕⊕◯ MODERATE [a b d]	We have moderate confidence that PNCS is associated with a 102% relative increase in predicting mortality PNCS probably predicts mortality
Correlation of FVC and CMAP amplitude № of participants: 281 (7 studies)	r 0.40 (0.23 to 0.55)	⊕⊕⊕◯ MODERATE [a b]	We have moderate confidence that CMAP is positively correlated with FVC CMAP levels on PNCS are probably positively correlated with FVC
Correlation of FVC and CMAP latency № of participants: 112 (3 studies)	r -0.23 (-0.42 to -0.02)	⊕⊕◯◯ LOW [a c]	We have low confidence that latency is negatively correlated with FVC Latency on PNCS may be negatively correlated with FVC

Table 2. GRADE table summary findings

CI: Confidence interval; HR: Hazard ratio; r: Correlation coefficient. GRADE Working Group grades of evidence: high certainty: we are very confident that the true effect lies close to that of the estimate of the effect; moderate certainty: we are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different; low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect. Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

Explanations:
a. Downgraded due to study limitations;
b. Downgraded due to inconsistency: large I^2;
c. Downgraded due to imprecision: optimal information size not met;
d. Upgraded due to large effect size

Discussion

Main findings

Overall, we found a low to moderate evidence suggesting that PNCS have a prognosis value in ALS disease. This conclusion is based on eight observational studies, enrolling a total of 604 patients with ALS.

PNCS probably predicts mortality in ALS. Only two of the eight included studies, enrolling 388 patients, reported hazard ratios for mortality. Our pooled analysis showed an approximately two-fold higher probability of death in patients with a CMAP amplitude below the 0.4mV cut-off. Significant statistical heterogeneity (I^2 55.9%) was found among these studies, which probably was driven by differences in methodological and clinical features between studies. One of them[1] (Bokuda *et al* 2014) had only a published abstract, which lead to a high risk of bias, due to a lack of information regarding the study design and the analysis reporting. Both studies used amplitude of CMAP for the survival analysis, which seems to be associated with a more accurate prediction of hypoventilation and survival than latency[31; 33]. A 0.4 mV cut-off was considered in both studies, which was reported in a previous study of the same authors of one of the two papers included [36]. Nevertheless, a 0.3 mV cut-off was purposed by other authors [15; 37], which probably leaded to a higher hazard ratio for mortality.

Although no single test has been shown to correlate well with respiratory failure in ALS[22], PFT are the most used measurements in clinical practise and research for pulmonary diseases. FVC was identified was the most relevant prognostic factor in ALS in most studies. Therefore, in this systematic review we looked at the correlation between, the FVC and PNCS parameters (amplitude and latency), in an alternatively way to evaluate its capacity to predict outcome in ALS patients. We found a moderate evidence that amplitude of CMAP is positively correlated with FVC and that latency may be negatively correlated with FVC. A significant heterogeneity (I^2 69,77%) was found among the studies enrolled in the correlation of amplitude of CMAP and FVC, which has probably driven by differences in methodological and clinical features between studies. For instance, the method and setting of outcome measurement was not the same in all participants in Jenkins *et al* 2016,[15] as the stimulation intensity of the CMAP record was different in some of the included patients in the study. It was not clear if the method and

setting of outcome measurement was the same in all participants in Singh *et al* 2011,[39] as the poor-quality of the spirograms in bulbar patients lead to a week of training by this subgroup of patients.

The other pulmonary function tests, such as maximal voluntary ventilation (MVV), maximal inspiratory (MIP) and expiratory (MEP) pressures, and nasal inspiratory pressure during sniff (SNIP) were not considered in this study as these tests have not show a consistent correlation with prognosis. MIP, despite being more sensitive to detect hypoventilation than FVC in ALS,[16] is negatively influenced by oro-facial paresis.[32] Besides that, it seems not being useful to follow patients over a long period time as it has a marked early decline then stabilizing (floor-effect). SNIP, despite being more suited for ALS patients with oro-facial paresis, it is only predictive of hypoventilation in spinal-onset patients.[24]

Limitations

The main limitations of this study were the heterogeneity of the purposes and methodology standards and reporting between the primary studies.

Clinical features of the studies, such as respiratory symptoms, ALSFRS-R, duration of the disease, type of onset, or NIV initiation, may contribute to the heterogeneity of the results. For instance, in Jenkins *et al* 2016, which was the study with the highest FVC and amplitude of CMAP correlation coefficient (0.69), patients presented a wide range of disease duration (2-102 months) and around 21% patients were using NIV at the time of the study, which may possibly influence the results.

Implications for research

Sub-group analysis was not performed due to a lack of data of the individual studies. However, it would be interesting to compare spinal and bulbar onset subgroups regarding the PCNS prognosis impact. This would be relevant mainly for bulbar subgroup, which has less compliance to PFT due to orofacial paresis, and therefore would be benefit more for PNCS.

It would be also relevant analyse the specific impact of PNCS in NIV initiation, as this is the intervention that has been shown that improves survival,[3] proportionally to duration of use,[27] as well as quality of life.[2; 3]

We opted for the prognosis value analysis of PNCS, as we considered the higher impact on the clinical practise. Despite this, the diagnosis accuracy of the test should be evaluate in a subsequent study.

Larger cohort studies with the complete follow-up, unbiased case selection and complete ascertainment of the possible confounders need to be performed in order to ascertain the capacity of PNCS in predicting ALS outcome.

Implications for practice

Further research is needed in order to validate PNCS as a biomarker of pulmonary function in ALS.

Including retrospective studies can be confounding due to the fact that it tends to include a large number of covariates that are related and can influence the biomarker.[8] For instance, the presence and the type of respiratory symptoms, the age of the patient, the duration of the disease, the type of onset, the functional status, the use of NIV are some of the potential confounders that should be considered in the data analysis.

Besides of that, the studies tend to include a multiplicity of clinical endpoints (i.e. FVC, MVV, MIP, MEP, SNIP, CMAP) in their analysis, which lead do higher level of false-positive associations. An analysis that prioritizes the relevant endpoint and uses a methodology that controls the family-wise error rate is necessary.[8]

Regarding the technique itself, like other electrophysiological techniques, PNCS is highly operator-dependent and its results depend on the quality of its performance and standardization of the technique is essential for the interpretation of the results.

In summary, improvements of standardization of the technique and using statistical methodologies that have inherent confounding and multiplicity in consideration are required to ascertain if PNCS can be used as biomarker in ALS patients in clinical practise.

Conclusion

Our study suggest that PNCS can probably be used as an additional marker of pulmonary function in ALS patients, but further research is needed.

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Appendixes

Appendix 1 – Fundamentação teórica

A esclerose lateral amiotrófica é uma doença neurodegenerativa rapidamente progressiva que envolve quer os neurónios motores superiores quanto inferiores. A maioria das mortes causadas por esta doença ocorrem como resultado de complicações respiratórias. Nesse sentido as provas de função respiratória servem como biomarcadores prognósticos nesta condição, essenciais para definir prognóstico, aconselhar os doentes e cuidadores e tomar decisões terapêuticas, tais como o início da ventilação não invasiva (NIV). Embora nenhum teste seja um *gold-standard* para avaliação da função respiratória nos doentes com ELA, dos parâmetros respiratórios, a capacidade vital forçada (CVF) é aquele que é utilizado com maior frequência na prática clínica e investigacional.

O estudo da condução do nervo frénico (PNCS) é um teste rápido, não-volitivo e acessível que pode ser utilizado para a avaliação da função respiratória nos doentes com ELA. Apresenta como vantagem em relação às provas de função respiratória, o facto de não depender da colaboração dos doentes, o que é útil em doentes com envolvimento bulbar, que apresentam paresia orofacial, e naquelas com distúrbios cognitivos, frequentes nesta doença. A latência e a amplitude da componente motora do potencial de ação são aferidos com este teste. Destes, a amplitude (CMAP) mostrou ser aquela que prediz hipoventilação (definida por $PaCO_2 > 45$ mmHg) e sobrevida nos doentes com ELA.

Posto isto, o nosso objetivo com este trabalho foi aferir o valor prognóstico do estudo da condução do nervo frénico na sua capacidade de predizer o risco de mortalidade, assim como a sua correlação com a capacidade vital forçada (CVF), nos doentes com ELA.

Conduzimos uma revisão sistemática de estudos observacionais que comparavam os resultados do nosso teste em estudo, PNCS, e o teste de referência, PFR, em adultos com diagnóstico definitivo ou provável de ELA, como definido pelos critérios *El Escorial*. Pesquisámos nas bases de dados MEDLINE (pela Ovid), EMBASE (pela Ovid), e Web of Science até Outubro de 2017. Dois revisores independentes reviram os títulos e resumos, os textos completos e fizeram a extração dos dados de prognóstico, utilizando o Covidence®. Considerámos como resultados de prognóstico: os *hazard ratios* para mortalidade para diferentes *cut-offs* de amplitude de CMAP, assim como a correlação

entre a FVC e a amplitude, e a entre o FVC e a latência do CMAP. O risco de viés foi avaliado utilizando a ferramenta *Quality In Prognosis Studies (QUIPS*). Para efetuar a meta-análise dos *hazard ratios*, utilizámos o método do inverso da variância para efeitos aleatórios. Para os resultados de medida de efeito de variáveis contínuas, transformámos o coeficiente de correlação na escala de *z* de Fisher, e efetuámos a meta-análise utilizando o método do inverso da variância, apresentando os resultados convertidos de novo a correlações. A heterogeneidade dos resultados foi avaliada com I^2. Foi considerado um valor de $p<0.05$ como estatisticamente significativo. Avaliámos a qualidade da evidência utilizando o *Grading of Recommendations Assessment, Development and Evaluation (GRADE)*, aplicado a estudos de avaliação prognóstica.

Um total de 365 referências (MEDLINE 210, EMBASE 170, Web of Science 55) foram obtidas pela pesquisa eletrónica. Após exclusão das referências repetidas, 233 foram obtidas para revisão por título e resumo. Destas, excluímos 204 referências. O texto completo de 29 referências foi examinado. Destes, 21 estudos foram excluídos. Oito estudos cumpriam os critérios de inclusão. Todos eles são estudos observacionais, incluindo um total de 604 participantes. A idade destes variou entre 58,2 e 61,5 anos, com uma duração de doença entre os 16,0 e 27,6 meses. O envolvimento bulbar variou entre 22,5% a 51,6%. Em dois dos estudos, os doentes iniciaram ventilação não-invasiva.

Na meta-análise, verificou-se que os doentes com amplitude de CMAP igual ou inferior a 0.4mV tinham uma probabilidade de morrer 2.021 vezes superior, durante o período estudado (IC 95%= 1.161 a 3.522; I^2 = 69.77%; 381 participantes). A amplitude de CMAP mostrou-se correlacionar positivamente com a CVF (coeficiente de correlação de 0.400; IC 95%= 0.226 a 0.550; I2= 69.77%; 381 participantes). Por outro lado, verificou-se uma correlação fraca negativa entre a latência de CMAP e a CVF (coeficiente de correlação de -0.235; IC 95% = 0.447 a -0.024; I^2=15.92%; 112 participantes). O risco de viés aferido pela ferramenta GRADE foi moderado a elevado, verificando maior risco de viés nos domínios *study attrition*, *study confounding* e *study analysis and reporting*. O grau de evidência aferido pelo GRADE baixo a moderado: moderado para o *hazard ratio* de mortalidade e correlação da amplitude de CMAP com a CVF; baixa para a correlação entre a latência de CMAP e a CVF.

Uma evidência moderada indica que o estudo da condução do frénico pode ser considerado um marcador adicional da função respiratória, nos doentes com ELA, mas que mais investigação é necessária.

Dos oitos estudos incluídos, apenas dois reportavam uma análise de mortalidade relacionada com o PNCS, o nosso *outcome* primário, sendo que um deles apenas o abstract está publicado, pelo que a escassez de dados quanto ao desenho do estudo e da análise dos dados. Quer para a mortalidade quer para a correlação entre a amplitude do CMAP e a CVF, constatou-se uma significativa heterogeneidade estatística (I^2 69,77% e 55,9%, respectivamente), provavelmente devida às diferenças nas características clínicas e metodológicas dos estudos incluídos.

Nenhum dos estudos incluiu possíveis fatores confundidores no desenho do estudo, tais como a presença ou ausência de sintomas respiratórios, o score de ALSFRS-R, a duração da doença, o tipo de início (espinhal ou bulbar), ou a iniciação de ventilação não invasiva. Para além do mais, a maioria dos estudos inclui uma miríade de variáveis respiratórias (tais como, volume ventilatório máximo, pressão inspiratória máxima, pressão expiratória máxima, pressão inspiratória nasal) que conduz a uma maior percentagem de associações de falsos-positivos. É necessária uma análise que priorize os *outcomes* e recorra a uma metodologia que reduza o erro do tipo I. Outro fator a ter em conta diz respeito à técnica em si. O estudo do nervo frénico é altamente operador-dependente e como tal os seus resultados dependem da qualidade da sua concretização. É necessária uma estandardização e difusão da técnica para que esta possa ser aplicada na prática clínica de forma regular.

Embora não tenha sido incluída neste estudo por falta de dados, a sub-análise de grupo, espinhal e bulbar, seria importante uma vez que os doentes bulbares, pela sua menor *compliance* com as PFR, beneficiariam do PNCS. Além disso seria relevante avaliar o impacto do PNCS na decisão de início de ventilação não-invasiva, uma vez que esta é uma intervenção que mostrou melhorar sobrevida e qualidade de vida. Do mesmo modo, a avaliação da acuidade diagnóstica do PNCS deveria ser avaliada num estudo subsequente.

Em suma, o nosso estudo sugere que o PNCS provavelmente pode ser utilizado como biomarcador adicional da função pulmonar nos doentes com ELA. Contudo, coortes de

maiores dimensões, com o seguimento completo, sem viés de seleção, com inclusão e devida análise dos possíveis fatores confundidores são necessários para a validação do PNCS como biomarcador.

Appendix 2 - MEDLINE search strategy

1. exp Phrenic Nerve/
2. phrenic$.tw.
3. or/1-2
4. exp Motor Neuron Disease/
5. (moto$1 neuron$1 disease$1 or moto? neuron$1 disease).ti,ab.
6. ((Charcot$1 adj5 syndrome$1) or (Lou Gehrig$1 adj5 syndrome$1)).ti,ab.
7. ((Charcot$1 adj5 disease) or (Lou Gehrig$1 adj5 disease)).ti,ab.
8. Amyotrophic Lateral Sclerosis.ti,ab.
9. or/4-8
10. and/3,9
11. (animals not (animals and humans)).sh.
12. 10 not 11

Appendix 3 - EMBASE search strategy

1. exp Phrenic Nerve/
2. phrenic$.tw.
3. or/1-2
4. exp Motor Neuron Disease/
5. (moto$1 neuron$1 disease$1 or moto$1 neuron$1 disease).ti,ab.
6. ((Charcot$1 adj5 syndrome$1) or (Lou Gehrig$1 adj5 syndrome$1)).ti,ab.
7. ((Charcot$1 adj5 disease) or (Lou Gehrig$1 adj5 disease)).ti,ab.
8. Amyotrophic Lateral Sclerosis.ti,ab.
9. or/4-8
10. and/3,9
11. nonhuman/ not human/
12. 10 not 11

Appendix 4 – Web of Science search strategy

1. TI=(phrenic) AND TI= (motor neuron) OR amyotrophic OR Charcot OR Lou Gehrig)